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(72) Inventor EDWARD WALTON

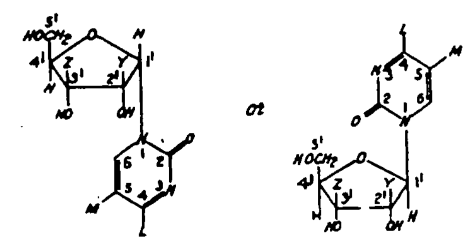
(54) SUBSTITUTED RIBOFURANOSYL PYRIMIDINE NUCLEOSIDES

(71) We, MERCK & Co. INC., a corporation duly organised and existing under the laws of the State of New Jersey, United States of America, of Rahway, New Jersey, United States of America, do hereby declare the invention for which we pray that a patent may be granted to us and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention is concerned with 2 - (or 3) - alkylribofuranosyl pyrimidine nucleosides and their preparation by reacting a 2, 3, 5 - tri - O - acyl - 2 - (or 3) - C - alkyl - D - ribofuranosyl halide with a chlorimercuric pyrimidine compound.

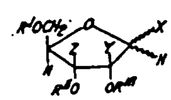
The 2 - C - methyl - D - ribofuranosyl halide starting materials are claimed in and may be prepared by methods described and claimed in the specification of our copending application No. 19293/67 (1187824). An alternative route which is applicable to the preparation of the 2 - alkyl - ribofuranosyl halides generally is set forth below. The 3 - alkyl - ribofuranosyl halide starting materials are claimed in and may be prepared by processes described and claimed in the specification of our copending application No. 49923/66 (1163102).

The novel branched-chain compounds of the present invention are the α- and β-anomers of nucleosides represented by formula (B):



α-anomer β-anomer

The starting materials claimed in the said copending applications are represented by formula (C):



(C)

In these formulae, L is a C_{1-5} alkoxy, hydroxy, amino or (C_{1-5} alkyl) - substituted amino radical; M is a hydrogen or halogen atom or a C_{1-5} alkoxy, hydroxy, amino, (C_{1-5} alkyl) - substituted amino, C_{1-5} alkyl, C_{1-5} alkyl) - halogenated alkyl radical; each of R′, R″ and R‴ is a hydrogen atom or a C_{1-6} alkanoyl, benzoyl or substituted benzoyl radical; X is a chlorine or bromine atom or a hydroxy, C_{1-5} alkoxy, C_{1-6} alkanoyl, benzoyl or substituted benzoyl radical and each of Y and Z is a C_{1-5} alkyl radical or a hydrogen

atom, provided that when Y is C_{1-3} alkyl Z is hydrogen, and when Y is hydrogen Z is C_{1-3} alkyl.

Typical of the alkanoyl groups are acetyl, propionyl and butyryl. The benzoyl group may be unsubstituted or substituted by C_{1-3} alkyl (e.g. it may be toluyl or xyloyl), C_{1-3} alkoxy such as methoxy or ethoxy, halogen such as chlorine or bromine or nitro.

Typical values of L and M are methoxy, ethoxy, propoxy, hydroxy, amino, methylamino, dimethylamino, ethylamino, diethylamino, propylamino and dipropylamino. Other typical values of M are hydrogen, methyl, ethyl, propyl, chlorine, bromine, iodine, fluorine and trifluoromethyl.

Compounds of formula (B) have demonstrated a variety of valuable utilities. Some of them have demonstrated antiviral activity. Some have been shown capable of inhibiting ribonucleic acid (RNA) synthesis, for example, acid-insoluble RNA synthesis, in Ehrlich ascites cells and KB cells. In in vitro tests, the growth of KB cells is markedly suppressed as is the incorporation of hypoxanthine into acid insoluble RNA. The compounds are therefore useful as antimetabolites as cell growth inhibitors and for the study of metabolism systems. They also demonstrate favourable cytotoxicity characteristics considered with their cell growth depression. In addition, they show a marked resistance to the action of adenosine deaminase, which means that such compounds can be expected to stay longer in the animal body so that their activity can be biologically useful.

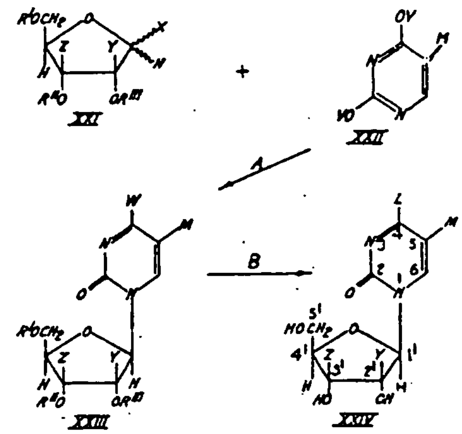
The nucleosides may also be converted to nucleotides by treatment with phosphoric acid derivatives in accordance with known techniques. As such, they are useful in a formulation of media for selective culturing of animal tissue cells. These nucleotides may also be useful in the study of nucleic acid metabolism.

Representative of the novel compounds obtained by the method of the present invention are the α and β forms of 1 - (2 - [or 3] - C methyl - D - ribofuranosyl) - 4 - methoxy - 2(1H) - pyrimidinone; 1 - (2 - [or 3] - C - methyl - D - ribofuranosyl) - 4 - ethoxy - 2(1H) - pyrimidinone; 1 - (2 - [or 3] - C - methyl - D - ribofuranosyl) - 4 - propoxy - 2(1H) - pyrimidinone; 1 - (2 - [or 3] - C - methyl - D - ribofuranosyl) - 4 - hydroxy - 5 - chloro - 2(1H) - pyrimidinone; 1 - (2 - [or 3] - C - methyl - D - ribofuranosyl) - 4 - amino - 5 - trifluoromethyl - 2(1H) - pyrimidinone; 1 - (2 - [or 3] - C - methyl - D - ribofuranosyl) - 4 - hydroxy - 5 - trifluoromethyl - 2 - (1H) - pyrimidinone; 1 - (2[or 3] - C - methyl - D - ribofuranosyl) - 4 - hydroxy - 5 - bromo - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - methyl - D - ribofuranosyl) - 4 - hydroxy - 5 - iodo - 2 - (1H) - pyrimidinone; 1 - (2[or 3] - C - methyl - D - ribofuranosyl) - 4 - hydroxy - 5 - fluoro - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - methyl - D - ribofuranosyl) - 4 - amino - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - methyl - D - ribofuranosyl) - 4 - methyl - amino - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - methyl - D - ribofuranosyl) - 4 - di - methylamino - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - methyl - D - ribofuranosyl) - 4 - diethylamino - 2(1H) - pyrimidone; 1 - (2[or 3] - C - methyl - D - ribofuranosyl) - 4, 5 - dimethoxy - 2(1H) - pyrimidinone; 1 - (2 - [or 3] - C - methyl - D - ribofuranosyl) - 4 - methoxy - 5 - chloro - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - methyl - D - ribofuranosyl) - 4 - methoxy - 5 - fluoro - 2(1H - pyrimidinone; 1 - (2[or 3] - C - methyl - D - ribofuranosyl) - 4 - hydroxy - 5 - methyl - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - methyl - D - ribofuranosyl) - 4 - hydroxy - 5 - ethyl - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - methyl - D - ribofuranosyl) - 4 - amino - 5 - methyl - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - methyl - D - ribofuranosyl) - 4 - amino - 5 - ethyl - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - methyl - D - ribofuranosyl) - 4 - methoxy - 5 - methyl - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - methyl - D - ribofuranosyl) - 4 - ethoxy - 5 - methyl - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - methyl - D - ribofuranosyl) - 4 - methoxy - 5 - ethyl - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - methyl - D - ribofuranosyl) - 4 - ethoxy - 5 - ethyl - 2(1H) - pyrimidinone; 1 - (2 - [or 3] - C - methyl - D - ribofuranosyl) - 4 - amino - 5 - bromo - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - methyl - D - ribofuranosyl) - 4 - amino - 5 - fluoro - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - methyl - D - ribofuranosyl) - 4 - hydroxy - 5 - amino - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - methyl - D - ribofuranosyl) - 4 - hydroxy - 5 - methyl - amino - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - methyl - D - ribofuranosyl) - 4 - hydroxy - 5 - dimethylamino - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - ethyl - D - ribofuranosyl) - 4 - methoxy - 2 -(1H) - pyrimidinone; 1 - (2[or 3] - C - ethyl - D - ribofuranosyl) - 4 - ethoxy - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - ethyl - D - ribofuranosyl) - 4 - propoxy - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - ethyl - D - ribofuranosyl) - 4 - hydroxy - 5 - chloro - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - ethyl - D - ribofuranosyl) - 4 - amino - 5 - trifluoromethyl - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - ethyl - D - ribofuranosyl) - 4 - hydroxy - 5 - trifluoromethyl - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - ethyl - D - ribofuranosyl) - 4 - hydroxy - 5 - bromo - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - ethyl - D - ribofuranosyl) - 4 - hydroxy - 5 - iodo - 2(1H) - pyrimidinone; 1 - (2

hydroxy - 5 - fluoro - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - ethyl - D - ribofuranosyl) - 4 - amino - 2 - (1H) - pyrimidinone; 1 - (2[or 3] - C - ethyl - D - ribofuranosyl) - 4 - methylamino - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - ethyl - D - ribofuranosyl) - 4 - dimethylamino - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - ethyl - D - ribofuranosyl) - 4 - diethylamino - 2(1H) - pyrimidinone; 1 - (2 - [or 3] - C - ethyl - D - ribofuranosyl) - 4, 5 - dimethoxy - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - ethyl - D - ribofuranosyl) - 4 - methoxy - 5 - chloro - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - ethyl - D - ribofuranosyl) - 4 - methoxy - 5 - fluoro - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - ethyl - D - ribofuranosyl) - 4 - hydroxy - 5 - methyl - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - ethyl - D - ribofuranosyl) - 4 - hydroxy - 5 - ethyl - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - ethyl - D - ribofuranosyl) - 4 - amino - 5 -methyl - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - ethyl - D - ribofuranosyl) - 4 - amino - 5 - ethyl - 2(1H) pyrimidinone; 1 - (2[or 3] - C - ethyl - D - ribofuranosyl) -4 - methoxy - 5 - methyl - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - ethyl - D - ribofuranosyl) - 4 - ethoxy - 5 - methyl - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - ethyl - D - ribofuranosyl) - 4 - methoxy - 5 - ethyl - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - ethyl - D - ribofuranosyl) - 4 - ethoxy - 5 - ethyl -2(1H) - pyrimidinone; 1 - (2[or 3] - C - ethyl - D - ribofuranosyl) - 4 - amino - 5 - bromo - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - ethyl - D - ribofuranosyl) - 4 - amino - 5 - fluoro - 2 - (1H) - pyrimidinone; 1 - (2[or 3] - C - ethyl - D - ribofuranosyl) - 4 - hydroxy - 5 - amino - 2(1H) - pyrimidinone; 1 - (2[or 3] - C - ethyl - D - ribofuranosyl) - 4 - hydroxy - 5 - methylamino - 2(1H) - pyrimidinone; and 1 - (2[or 3] - C - ethyl - D - ribofuranosyl) - 4 - hydroxy - 5 - dimethylamino - 2(1H) - pyrimidinone.

Both α and β anomers of those compounds of the present invention in which M is a hydrogen atom and w is an alkoxy radical are prepared by reacting a 2, 4 - dialkoxypyrimidine with a 2, 3, 5 - tri - O - acyl - 2(or 3) - C - (C_{1-5} alkyl) - D - ribofuranosyl halide to form a 1 - (2, 3, 5 - tri - O - acyl - 2[or 3] - C - (C_{1-5} alkyl) - D - ribofuranosyl) - 4 - alkoxy - 2 (1H) - pyrimidinone. These intermediate compounds are then reacted with ammonia, or a primary or secondary amine to produce compounds (XXIV) wherein L is an amino or substituted amino. The reaction product from Step A may also be hydrolysed under acidic or basic conditions to produce compounds (XXIV) where L is hydroxy.

The reaction is illustrated by the follow-



where

L, M, Y and Z are as previously defined;

X is a halogen in either the α or β configuration, or a combination of both;

V is C_{1-5} alkyl;

W is C_{1-5} alkoxy or hydroxy; and

R′, R″ and R‴ are acyl groups.

Examples of acyl groups are alkanoyl such as acetyl, propionyl or butyrol; benzoyl; and benzoyl substituted by lower alkyl, alkoxy, halo or nitro groups. Solvents may be lower alkanols.

More specifically, the process of the present invention involves, in Step A, the reaction of an excess of a 2, 4 - di(C_{1-5} alkoxy)pyrimidine with a 2, 3, 5 - triacyl - 2 - (or 3) - C - (C_{1-5} alkyl) - D - ribofuranosyl halide at a temperature range of from 5° C. to 120° C., and preferably 25° C. to 60° C. until reaction is complete. In this step, the reaction is carried out in an appropriate solvent. The selection of the solvent is not important as long as it is an inert solvent. Examples of such solvents are methylene chloride, benzene, diethyl ether, dibutyl ether, dioxane, tetrahydrofuran and cyclohexane. The preferred solvent is methylene chloride. The reaction to produce the α and β-anomers (XIII) is normally complete in a few hours to several days, depending on the reaction temperature and the reactivity of the halogenose.

The reaction product from Step A, where W is C_{1-5} alkoxy, is then reacted with ammonia, or a C_{1-5} alkylamine or di(C_{1-5} alkyl) amine such as methylamine, ethylamine, propylamine, or dimethylamine in Step B in an appropriate solvent at about the same temperature range as in Step A, to produce compound XXIV in which L is amino, C_{1-5} alkylamino or di(C_{1-5} alkyl)amino.

The novel 2(1H)-pyrimidinone nucleosides can also be obtained by condensing the halo sugar reactant with a mercury complex of an appropriately substituted pyrimidine.

compounds of the present invention, an additional step may be required. For example, the 5 - halo - derivatives are obtained by halogenating the 1 - (2[or 3] - lower - alkyl - D - ribofuranosyl) - 4 - hydroxy - 2(1H) - pyrimidinone by methods known in the art for halogenating 1 - (D - ribofuranosyl) - 4 - hydroxy - 2(1H) - pyrimidinone. The resulting 1 - (2[or 3] - lower alkyl - D - ribo - furanosyl) - 5 - halo - 4 -hydroxy - 2(1H) - pyrimidinone is then converted to the corresponding 1 - (2[or 3] - lower - alkyl - D - ribofuranosyl) - 4 - hydroxy - 5 - amino - 2(H) - pyrimidinone by treatment with ammonia or a primary or secondary amine as heretofore described. For example, 1 - (2 - C - methyl) - D - ribofuranosyl) - 4 - hydroxy - 5 - amino - 2(1H) - pyrimidinone is preferably obtained by brominating 1 - (2 - methyl) - D - ribofuranosyl - 4 - hydroxy - 2(1H) - pyrimidinone, and then reacting the resulting 1 - (2 - methyl) - D - ribofuranosyl - 4 - hydroxy - 5 - bromo - 2(1H) - pyrimidinone with ammonia.

The following examples illustrate methods of carrying out the processes of the present invention. The word "Dowex" is a trade mark.

Example 1

2′ - C - methyluridine

A solution of 20 mg. (0.035 mmole) of 1 - (2, 3, 5 - tri - O - benzoyl - 2 - C - methyl - β - D - ribofuranosyl)uracil is added to a solution prepared from 1.6 mg. (0.07 mg. atom) of sodium and 2 ml. of methanol. The mixture is refluxed for 30 minutes and concentrated to dryness. The residue is dissolved in 2 ml. of water and washed with three 1-ml. portions of ether. Concentration of the water layer gives a residue of 2′ - C - methyluridine.

Similarly, the corresponding 2′ - C - ethyl - uridine, 3′ - C - methyluridine, or 3′ - C - ethyluridine is obtained when in place of the 1 - (2′, 3′, 5′ - tri - O - benzoyl - 2 - C - methyl - β - D - ribofuranosyl)uracil, the 1 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - ethyl - β - D - ribofuranosyl)uracil, 1 - (2′, 3′, 5′ - tri - O - benzoyl - 3′ - C - methyl - β - D - ribofuranosyl)uracil, or 1 - (2′, 3′, 5′ - tri - O - benzoyl - 3′ - ethyl - β - D - ribofuranosyl)uracil is used as the starting material.

Example 2

2′ - C - Methylcytidine

From 1 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - methyl - β - D - ribofuranosyl) - 4 - methoxy - 2(1H) - pyrimidinone

A solution of 1.0 g. (1.7 millimoles) of 1 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - methyl - β - D - ribofuranosyl) - 4 - methoxy - 2(1H) - pyrimidinone in 14 ml. is heated in a sealed tube at 100° C. for 17 hours. The solvent is removed at reduced pressure, and the residue is dissolved in 30 ml. of water. A small amount of benzamide is removed by filtration and the aqueous filtrate is extracted with five 50-ml portions of ether. The aqueous solution is concentrated at reduced pressure at a temperature of less than 40° C., and the residue (0.5 g.) is crystallized from methanol. Recrystallization from methanol gives 394 mg. (90%) of 2′ - C - methylcytidine, m.p. 243—245° C. (transition between 140 and 170° C.), $[\alpha]_D$ +91° (c, 1, water).

Similarly, 2′ - C - ethylcytidine, 3′ - C - methylcytidine, or 3′ - C - ethylcytidine is obtained when in place of the 1 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - methyl - β - D - ribofuranosyl) - 4 - methoxy - 2(1H) - pyrimidinone the corresponding 1 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - ethyl - β - D - ribofuranosyl) - 4 - methoxy - 2(1H) - pyrimidinone, 1 - (2′, 3′, 5′ - tri - O - benzoyl - 3′ - C - methyl - β - D - ribo - furanosyl) - 4 - methoxy - 2(1H) - pyrimidi - none, or 1 - (2′, 3′, 5′ - tri - O - benzoyl - 3′ - C - ethyl - β - D - ribofuranosyl) - 4 - methoxy - 2(1H) - pyrimidinone is used as the starting material.

Example 3

2′ - C - Methylcytidine

From 1 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - methyl - β - D - ribofuranosyl) - 4 - acetamidopyrimidine

2, 3, 5 - tri - O - benzoyl - 2 - C - methyl - D - ribofuranosyl chloride in 75 ml. of dry xylene is added to a suspension of 527 mg. (2 millimoles) of N - acetylcytosine mercury in 75 ml. of dry xylene at 100° C. The mixture is heated at the reflux temperature and stirred for 30 minutes. The reaction solution is concentrated to 35 ml., cooled, and treated with 175 ml. of petroleum ether; and then further cooled in an ice-bath. The precipitated solid is removed, dissolved in 100 ml. of chloroform, and then washed with three 40-ml. portions of 30% potassium iodide solution and two 40-ml. portions of water. The chloroform solution is concentrated, and the residue (1.2 g.) is chromatographed on a short column of 40 g. of silica gel in mixtures of chloroform and ethyl acetate (1:1). The eluant is monitored by thin layer chromatography (tlc) on silica plates in mixtures of chloroform and ethyl acetate (1:1). The first several fractions contain two reaction products of R_f (tlc) 0.8 and 0.96. Later fractions contain a reaction product showing an R_f (tlc) of 0.23. These fractions are combined and concentrated to give 100 mg. (13% based on N - acetyl - cytosine mercury) of 1 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - methyl - β - D -

pyrimidinone as a glass; $[\alpha]_D$ −46° (c 0.86, chloroform), λ_{max}^{EtOH} mμ ($\epsilon \times 10^{-3}$): 231 (43.0), 273 inf. (8.0), 2825 (7.3), 300 (6.1).

The first fractions to be removed from the chromatographed column are combined and concentrated at reduced pressure. The residue (1.05 g.) is then rechromatographed on a short column of 40 g. of silica gel in mixtures of benzene and ethyl acetate (19.1). The first several fractions yield 200 mg. of several by-products, followed by fractions containing 600 mg. of product. Crystallization twice from methanol gives 400 mg. (52%) of 2 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - methyl - β - D - ribofuranosyloxy) - 4 - acetamido - pyrimidine: m.p. 99—100° C., $[\alpha]_D$ +30.2° (c 1, chloroform), λ_{max}^{EtOH} mμ ($\epsilon \times 10^{-3}$): 230 (49.5), 274 (14.5).

A solution of 100 mg. (0.17 mmole) of 2 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - methyl - D - ribofuranosyloxy) - 4 - acetamidopyrimidine in 20 ml. of dry xylene containing 180 mg. (0.5 millimole) of mercuric bromide is refluxed for 4 hours. The hot xylene solution is filtered, and the filtrate is concentrated at reduced pressure. The residue is added to 20 ml. of chloroform, and some insoluble material is removed. The chloroform solution is washed with three 15-ml. portions of 30% potassium iodide solution and three 15-ml. portions of water. Concentration of the chloroform layer gives 80.5 mg. of a residual glass. The residue is chromatographed on a short silica gel column in benzene-ethyl acetate (1:1). After removal of a by-product, fractions containing the desired 1 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - methyl - β - D - ribofuranosyl) - 4 - acetamido - 2(1H) - pyrimidinone are obtained.

A solution of 47 mg. (0.08 millimole) of 1 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - methyl - β - D- ribofuranosyl) - 4 - acetamido - pyrimidine in 4 ml. of methanol saturated with ammonia at 0° C. is heated in a sealed tube at 100° C. for 17 hours.. The solution is concentrated at reduced pressure, and the residue is dissolved in 10 ml. of water and extracted with four 2-ml. portions of chloroform. The aqueous layer is freeze-dried. The residue is then redissolved in 10 ml. of water and extracted with five 10-ml portions of ether. Concentration of the aqueous layer at reduced pressure gives 17 mg. of 2′ - C - methylcytidine having properties identical with those of 2′ - C - methylcytidine prepared in Example 2.

Similarly, the corresponding 2′ - C - ethylcytidine, 3′ - C - methylcytidine, or 3′ - C - ethylcytidine is obtained when in place of the 2, 3, 5 - tri - O - benzoyl - 2 - C - methyl - D - ribofuranosyl chloride starting material in the above procedure there are used equivalent amounts of 2, 3, 5 - tri - O - benzoyl - 2 - C - ethyl - D - ribofuranosyl chloride, 2, 3, 5 - tri - O - benzoyl - 3 - C - methyl - D - ribofuranosyl chloride, and 2, 3, 5 - tri - O - benzoyl - 3 - C - ethyl - D - ribofuranosyl chloride.

EXAMPLE 4

1 - (3′ - C - Methyl - β - D - ribo - furanosyl)cytosine, i.e. 3′ - C - methyl - cytidine, and 1 - (3′ - C - methyl - α - D - ribofuranosyl)cytosine

A mixture of 4.08 milimoles of 2, 3, 5 - tri - O - benzoyl - 3 - C - methyl - α(and β) - D - ribofuranosyl bromide and 1.3 g. (9.27 millimoles) of 2, 4 - dimethoxypyrimidine in 75 ml. of dry methylene chloride is kept at room temperature for 5 days. Thin layer chromatography on alumina in chloroform-benzene (3:1) shows zones (visualized with iodine vapor) at R_f 0.2 (α - anomer of product), 0.6 (β-anomer of product), 0.8 (unreacted pyrimidine). The reaction mixture is diluted with 50 ml. of methylene chloride, and then extracted with three 30-ml. portions of cold 5% hydrochloric acid and 30 ml. of cold 5% potassium hydrogen carbonate. The methylene chloride solution is dried over anhydrous magnesium sulfate and concentrated to dryness. The residue (2.53 g.) is chromatographed on 50 g. of acid-washed alumina, eluting first chromatographed on 50 g. of alumina (acid-washed) eluting with benzene-chloroform (4:1), then with benzene - chloroform (1:4), and finally with chloroform. Fractions containing the (β-anomer) are pooled and concentrated. The residue is crystallized from a mixture of benzene and petroleum ether (1:1), to give a total of 1.07 g. (45%) of 1 - (2′, 3′, 5′ - tri - O - benzoyl - 3′ - C - methyl - β - D - ribo - furanosyl) - 4 - methoxy - 2(1H) - pyrimidi - none: m.p. 84—90° C.; $[\alpha]_D$ −76° (c, 1 in $CHCl_3$); λ_{max}^{MeOH} mμ ($\epsilon \times 10^{-3}$)—230 (43.4), 275 (9.4), 280 (8.6). Later-column fractions containing the α-anomer of the product are pooled and concentrated. Crystallization of the residue (120 mg.) gives purified 1 - (2′, 3′, 5′ - tri - O - benzoyl - 3′ - C - methyl - α - D - ribofuranosyl) - 4 - meth - oxy - 2(1H) - pyrimidinone: m.p. 206—209° C.; $[\alpha]_D$ −180° (c, 0.5 in $CHCl_3$); λ_{max}^{MeOH} mμ ($\epsilon \times 10^{-3}$—229 (38.0), 275 (9.3), 280 (8.6).

A mixture of 500 mg. (0.856 mmole) of 1 - (2′, 3′, 5′ - tri - O - benzoyl - 3′ - C - methyl - β - D - ribofuranosyl) - 4 - methoxy - 2(1H) - pyrimidinone in 7.5 ml. of methanol, previously saturated with ammonia at 0° C., is shaken for 16 hours at 100° C. in a sealed tube. The contents of the tube are concentrated, and the residual oil (1.07 g.) is dissolved in 50 ml. of water. The aqueous mix-

ture is extracted with three 30-ml. portions of ether to remove benzamide. The water solution is concentrated to dryness and the product crystallizes. The solid is dissolved in methanol, filtered and concentrated. On cooling 201 mg. (92%) of 3′ - C - methyl - cytidine is obtained: m.p. 235—238° C.; $[\alpha]_D$ +4° (c, 0.5 in H_2O); $\lambda^{H_2O}_{max}$ mμ (ε× 10^{-3})—pH 1—212.5 (10.6), 279 (12.9)—pH 7—232.5 (8.1), 271 (8.9)—pH 13—230 (8.2), 271 (8.9).

Similarly, the corresponding 3′ - C - ethylcytidine, the 2′ - C - methylcytidine or the 2′ - C - ethylcitidine is obtained when in place of the 2, 3, 5 - tri - O - benzoyl - 3 - C - methyl - α(and β) - ribofuranosyl bromide starting material an equivalent amount of the appropriate 3 - C - ethyl, 2 - C - methyl, or 2 - C - ethyl starting material is used.

A mixture of 50 mg. (0.085 mmole) of 1 - (2′, 3′, 5′ - tri - O - benzoyl - 3′ - C - methyl - α - D - ribofuranosyl) - 4 - meth - oxy 2(1H) - pyrimidinone and 3 ml. of methanol, previously saturated with ammonia at 0° C, is shaken for 16 hours at 100° C. in a sealed tube. The contents of the tube are concentrated to dryness and the residue (50 mg.) is dissolved in 4 ml. of water. The aqueous solution is extracted with three 3-ml. portions of ether. The aqueous layer is filtered and concentrated to a solid residue. Crystallization of the residue from 2.5 ml. of methanol gives about 20 mg. of 1 - (3′ - C - methyl - α - D - ribofuranosyl) - cytosine: m.p. 250—258° C.

Similarly, the corresponding 1 - (2 - C - methyl) - α - D - ribofuranosyl)cytosine, 1 - (2 - C - ethyl) - α - D - ribofuranosyl) cytosine, or 1 - (3 - C - ethyl) - α - D - ribo - furanosyl)cytosine is obtained when in place of the 1 - (2, 3, 5 - tri - O - benzoyl - 3 - C - methyl - α - D - ribofuranosyl - 4 - methoxy - 2(1H) - pyrimidinone in the above procedure there is used an equivalent amount of the appropriate 2 - C - methyl, 2 - C - ethyl or 3 - C - ethyl starting material.

Example 5

Step A

1 - (2, 3, 5 - Tri - O - benzoyl - 2 - C - methyl - β - D - ribofuranosyl) - 5 - fluoro - 4 - methoxy - 2(1H) - pyrimidinone

A solution of 4.8 g. (9.7 millimoles) of 2, 3, 5 - tri - O - benzoyl - 2 - C - methyl - β - D - ribofuranosylchloride in 18 ml. of dry toluene is added to 3.5 g. (22.2 millimoles) of 2, 4 - dimethoxy - 5 - fluoropyrimidine and the mixture is refluxed for 96 hours. The toluene solution is concentrated, and the residue is dissolved in 100 ml. of ether and extracted with three 50-ml. portions of 4 N hydrochloric acid, three 50-ml. portions of saturated sodium hydrogen carbonate, and finally with water. The ethereal solution is concentrated, and the residue (6.2 g.) is chromatographed on 150 g. of silica gel in a 19.1 mixture of benzene and ethyl acetate. After the elution of several by-products (R_f 0.8, 0.7, 0.6, 0.5 and 0.39—thin layer chromatography on silica gel in a 19:1 mixture of benzene and ethyl acetate, fractions containing a total of 3.9 g. of product (R_f 0.23—tlc) are obtained. Crystallization from 5 ml. of benzene and 50 ml. of ether affords 3.2 g. (55%) of 1 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - methyl - β - D - ribofuranosyl) - 5 - fluoro - 4 - methoxy - 2(1H) - pyrimidinone: m.p. 157—159° C.; $[\alpha]_D$ −14°, $[\alpha]_{578}$ −14° (c 1, chloroform); λ^{EtOH}_{max} mμ (ε× 10^{-3}), 229 (49.4), 277 (8.9), 283 (9.0), 293 inf. (6.4).

Anal. Calcd for $C_{32}H_{27}FN_2O_9$:
C, 63.79; H, 4.52; F, 3.15; N, 4.65.
Found:
C, 63.91; H, 4.34; F, 2.80; N, 4.35.

In accordance with the above procedure but replacing the 2, 4 - dimethoxy - 5 - fluoropyrimidine with an equivalent amount of 2, 4 - dimethoxy - 5 - trifluoromethyl-pyrimidine, or 2, 4 - dimethoxy - 5 - methyl-pyrimidine there is obtained the corresponding 1 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - methyl - β - D-ribofuranosyl) - 5 - trifluoromethyl - 4 - methoxy - 2(1H) - pyrimidinone, or 1 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - methyl - β - D - ribofuranosyl) - 5 - methyl - 4 - methoxy - 2(1H) - pyrimidinone.

Step B

5 - Fluoro - 1 - (2′ - C - methyl - β - D - ribofuranosyl) - cytosine, i.e. 5 - fluoro - 2′ - C - methylcytidine

A solution of 80 mg. (0.13 millimole) of 1 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - methyl - β - D -ribofuranosyl) - 5 - fluoro - 4 - methoxy - 2(1H) - pyrmidinone in 7 ml. of methanol, saturated with ammonia at 0°C., is heated at 100°C. in a sealed tube for 18 hours. The reaction solution is concentrated at reduced pressure, and the residue is dissolved in 10 ml. of water and extracted with three 5-ml. portions of ether. The aqueous phase is concentrated at reduced pressure and the residue, when crystallized from 0.2 ml. of methanol plus 0.01 ml. of ether, affords 24 mg. (67%) of 5 - fluoro - 2′ - C - methylcytidine: m.p. 247—249°C.; R_f 0.78—tlc on cellulose in water; $[\phi]_{400}$ +1200°, $[\phi]_{302}$ +15,700° (pk), $[\phi]_{281}$ 0°, $[\phi]_{255}$ −18,700° (tr), $[\phi]_{242}$ −16,300° (pk), $[\phi]_{233}$ −17,700° (tr), $[\phi]_{219}$ 0°; $\lambda^{H_2O}_{max}$ mμ (ε× 10^{-3}): pH 1—24 (9.7), 292 (11.1)—pH 7—2.3 (8.9), 238 (7.7), 282.5 (8.0)—pH 13—237 (7.7), 283 (8.1)

Anal. Calcd. for $C_{10}H_{14}FN_3O_5$:
C, 43.63; H, 5.13; N, 15.27.
Found: C, 43.38; H, 5.25; N, 14.98.

The corresponding 5 - trifluoromethyl - 2' - C - methyl - cytidine is obtained when an equivalent amount of 1 - (2', 3', 5 - tri - O - benzoyl - 2' - C - methyl - β - D - ribofuranosyl) - 5 - trifluoromethyl - 4 - methoxy - 2(1H) - pyrimidinone is used in place of 1 - (2', 3', 5' - tri - O - benzoyl - 2' - C - methyl - β - D - ribofuranosyl) - 5 - fluoro - 4 - methoxy - 2(1H) - pyrimidinone.

EXAMPLE 6

5 - Fluoro - 1 - (2 - C - methyl - β - D - ribofuranosyl)uracil, i.e. 5 - fluoro - 2' - C - methyluridine

A suspension of 602.5 mg. (1.0 mmole) of 1 - (2', 3', 5' - tri - O - benzovl - 2' - C - methyl - β - D-ribofuranosyl) - 5 - fluoro - 4 - methoxy - 2(1H) - pyrimidinone in 20 ml. of methanol is treated with 160 mg. (4.0 millimoles) of sodium hydroxide and 2 ml. of water. The mixture is refluxed for 45 minutes and the solution is concentrated at reduced pressure. The residue is dissolved in 20 ml. of water and small portions of Dowex 50×4 (H+) resin is added until the pH of the solution is 4.0. The resin and precipitated benzoic acid is removed and washed well with water. The combined filtrates are extracted with six 25-ml. portions of ether. The aqueous layer is concentrated at reduced pressure, and the residue (300 mg.) in 5 ml. of methanol is treated with 1 ml. of ether. The precipitated solid is removed, and the filtrate is concentrated to 0.3 ml and kept at 5°C. for 18 hours. The solid (107 mg., m.p. 196—205°C.) obtained, when recrystallized from 0.5 ml. of methanol and 0.5 ml. of ether affords 74 mg. (27%) of 5 - fluoro - 2' - C - methyluridine: m.p. 205—207°C.

EXAMPLE 7

5 - Bromo - 2' - methyluridine

A solution of 45.6 mg. (0.2 millimole) of 2' - methyluridine in 0.4 ml. of water is treated dropwise with a solution of bromine in water until a pale yellow color persists. Nitrogen is blown through the solution to remove excess of bromine, and the solution is lyophylized. The residual 1 - (2' - methyl - β - D - ribofuranosyl) - 4 - hydroxy - 5,6 - dibromo - 2(1, 5 , 6 - H)pyrimidone is dissolved in 1.5 ml. of ethanol. The solution is refluxed and hydrogen bromide is evolved. An ultraviolet absorption maximum at 282 mμ is generated during the heating period. The solution is concentrated at reduced pressure and the residual oil dissolved in water and washed with two 1-ml. portions of ether. The aqueous layer is concentrated to dryness. The product is dissolved in 2 ml. of water and treated with 30 mg. of decolorizing carbon. After removal of the carbon, the colorless aqueous solution is concentrated to dryness. Methanol is removed from the residue (53.5 mg.) at reduced pressure several times to eliminate the last traces of water. Trituration of the residue with ether affords 5 - bromo - 2' - methyluridine.

In accordance with the above procedure, but starting with 2' - ethyl -, 3' - methyl -, or 3' - ethyluridine there is obtained 2' - ethyl -, 3' - methyl -, or 3' - ethyl - 5 - bromo - uridine.

EXAMPLE 8

2' - C - Methyl - 5 - methylaminouridine

A solution of 3.9 g. (12 millimoles) of 5 - bromo - 3' - C - methyluridine in 40 ml. of anhydrous liquid methylamine is heated at 80°C. for 18 hours in a sealed tube. The amine is evaporated and the residue is dissolved in water and added to a column of 400 ml. of Dowex 50W×4 (H+). The column is washed well with distilled water to remove neutral, ultraviolet absorbing materials and the product is eluted with 0.5 N ammonium hydroxide. Concentration of the ammonium hydroxide eluant gives a residue of 2' - C - methyl - 5 - methylaminouridine.

If in the above procedure the 5 - bromo - 2' - C - methyluridine is replaced by 5 - bromo - 2' - C - ethyluridine, 5 - bromo - 3' - C - methyluridine, or 5 - bromo - 3' - C - ethyluridine there is obtained 2' - C - ethyl - 5 - methylaminouridine, 3' - C - methyl - 5 - methylaminouridine, or 3' - C - ethyl - 5 - methylaminouridine.

When in the above procedure the methylamine is replaced by ethylamine, dimethylamine, or methanol saturated at 0°C. with ammonia, there is obtained 2' - C - methyl - 5 - ethylaminouridine, 2' - C - methyl - 5 - dimethylaminouridine, or 2' - C - methyl - 5 - aminouridine.

EXAMPLE 9

2' - C - Methyl - 5 - trifluoromethyluridine

A solution of 1 g. of 1 - (2', 3', 5' - tri - O - benzoyl - 2' - C - methyl - β - D - ribofuranosyl) - 4 - methoxy - 5 - trifluoromethyl - 2(1H) - pyrimidinone in 50 ml. of methanol containing 1 ml. of concentrated hydrochloric acid is kept at 25°C. for several days. The solution is concentrated to dryness and a residue containing 2' - C - methyl - 5 - trifluoromethyluridine is obtained.

When the 1 - (2', 3', 5' - tri - O - benzoyl - 2 C - methyl - β - D - ribofuranosyl) - 4 - methoxy - 5 - trifluoromethyl - 2(1H) - pyrimidinone used above is replaced by 1 - (2', 3', 5' - tri - O - benzoyl - 3' - C - methyl - β - D-ribofuranosyl) - 4 - methoxy - 5 - trifluoromethyl - 2(1H) - pyrimidinone, 1 - (2', 3', 5' - tri - O - benzoyl - 2 - C - ethyl - β - D - ribofur-

anosyl) - 4 - methoxy - 5 - trifluoromethyl - 2(1H) - pyrimidinone, or 1 - (2′, 3′, 5′ - tri - O - benzoyl - 3′ - C - ethyl - β - D - ribofuranosyl) - 4 - methoxy - 5 - trifluoromethyl - 2(1H) - pyrimidinone there is obtained 3′ - C - methyl - 5 - trifluoromethyluridine, 2′ - C - ethyl - 5 - trifluoromethyluridine, or 3′ - C - ethyl - 5 - trifluoromethyluridine respectively.

Example 10

Step A

1 - (2′, 3′, 5′ - Tri - O - benzoyl - 2′ - C - methyl - β - D - ribofuranosyl) - 5 - fluoro - 4 - methoxy - 2(1H) - pyrimidone

A solution of 4.8 g. (9.7 millimoles) of 2, 3, 5 - tri - O - benzoyl - 2 - C - methyl - β - D - ribofuranosyl chloride in 18 ml. of dry toluene is added to 3.5 g. (22.2 millimoles) of 2,4 - dimethoxy - 5 - fluoropyrimidine and the mixture is refluxed for 96 hours. The toluene solution is concentrated and the residue is dissolved in 100 ml. of ether and extracted with three 50-ml. portions of 4 N hydrochloric acid and three 50-ml. portions of saturated sodium hydrogen carbonate and finally with water. The ethereal solution is concentrated and the residue (6.2 g.) is chromatographed on 150 g. of silica gel in a 19:1 mixture of benzene and ethyl acetate. After the elution of several by-products (R_f 0.8, 0.7, 0.6, 0.5 and 0.39—thin layer chromatography on silica gel in a 19:1 mixture of benzene and ethyl acetate, fractions containing 3.9 g. of product (R_f 0.23—tlc) are obtained. Crystallization from 5 ml. of benzene and 50 ml. of ether gives 3.2 g (55%) of 1 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - methyl - β - D - ribofuranosyl) - 5 - fluoro - 4 - methoxy - 2(1H) - pyrimidinone: m.p. 157—159°C; $[\alpha]_D$ −14°, $[\alpha]_{578}$ −14° (c 1, chloroform); λ_{max}^{EtOH} mμ ($\epsilon \times 10^{-3}$), 229 (49.4), 277 (8.9), 283 (9.0), 293 infl. (6.4).

Anal. Calcd. for $C_{32}H_{27}FN_2O_9$:

Found:

C, 63.79; H, 4.52; F, 3.15; N, 4.65

C, 63.91; H, 4.34; F, 2.80; N, 4.35

When the 2, 3, 5 - tri - O - benzoyl - 2 - C - methyl - β - D - ribofuranosyl chloride in the above procedure is replaced by 2, 3, 5 - tri - O - benzoyl - 2 - C - ethyl - β - D - ribofuranosyl chloride, 2, 3, 5 - tri - O - benzoyl - 3 - C - ethyl - β - D - ribofuransoyl chloride, or 2, 3, 5 - tri - O - benzoyl - 3 - C - ethyl - β - D - ribofuranosyl chloride there is obtained, respectively, 1 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - ethyl - β - D - ribofuranosyl) - 5 - fluoro - 4 - methoxy - 2(1H) - pyrimid-one, 1 - (2′, 3′, 5′ - tri - O - benzoyl - 3′ - C - methyl - β - D - ribofuranosyl) - 5 - fluoro - 4 - methoxy - 2(1H) - pyrimidone, or 1 - (2′, 3′, 5′ - tri - O - benzoyl - 3′ - C - ethyl - β - D - ribofuranosyl) - 5 - fluoro - 4 - methoxy - 2(1H) - pyrimidinone.

Step B

5 - Fluoro - 1 - (2′ - C - methyl - β - D - ribofuranosyl)cytosine i.e., 5 - fluoro - 2′ - C - methylcytidine

A solution of 80 mg. (0.13 millimole) of 1 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - methyl - β - D - ribofuranosyl) - 5 - fluoro - 4 - methoxy - 2(1H) - pyrimidinone in 7 ml. of methanol, saturated with ammonia at 0°C., is heated at 100°C. in a sealed tube for 18 hours. The reaction solution is concentrated at reduced pressure and the residue is dissolved in 10 ml. of water and extracted with three 5-ml. portions of ether. The aqueous phase is concentrated at reduced pressure and the residue, when crystallized from 0.2 ml. of methanol plus 0.01 ml. of ether, gives 24 mg. (67%) of 5 - fluoro - 2′ - C - methylcytidine: m.p. 247—249°C.; R_f 0.78—thin layer chromatography on cellulose in water; $[\phi]_{400}$ +1200°, $[\phi]_{302}$ +15,700° (pk), $[\phi]_{281}$ 0°, $[\phi]_{255}$ −18,700° (tr), $[\phi]_{242}$ −16,300° (pk), $[\phi]_{233}$ −17,700° (tr), $[\phi]_{219}$ 0°; $\lambda_{max}^{H_2O}$ nμ ($\epsilon \times 10^{-3}$): pH 1—214 (9.7), 292 (11.1)—pH 7—213 (8.9), 238 (7.7), 282.5 (8.0)—pH 13—237 (7.7), 283 (8.1).

Anal. Calcd. for $C_{10}H_{14}FN_3O_5$:

Found:

C, 43.63; H, 5.13; N, 15.27

C, 43,38; H, 5.25; N, 14.98.

When the 1 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - methyl - β - D - ribofuranosyl) - 5 - fluoro - 4 - methoxy - 2(1H) - pyrimidinone in the above procedure is replaced by an equivalent amount of 1 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - ethyl - β - D - ribofuranosyl) - 5 - fluoro - 4 - methoxy - 2(1H) - pyrimidinone, 1 - (2′, 3′, 5′ - tri - O - benzoyl - 3′ - C - methyl - β - D - ribofuranosyl) - 5 - fluoro - 4 - methoxy - 2(1H) - pyrimidinone, or 1 - (2′, 3′, 5′ - tri - O - benzoyl - 3′ - C - ethyl - β - D - ribofuranosyl) - 5 - fluoro - 4 - methoxy - 2(1H) - pyrimidinone there is obtained respectively 5 - fluoro - 2′ - C - ethylcytidine, 5 - fluoro - 3′ - C - methylcytidine, or 5 - fluoro - 3′ - C - ethylcytidine.

Example 11

5 - Fluoro - 1 - (2 - C - methyl - β - D - ribofuranosyl)uracil, i.e. 5 - Fluoro - 2′ -

A suspension of 602.5 mg. (1.0 mmole) of 1 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - methyl - β - D - ribofuranosyl) - 5 - fluoro - 4 - methoxy - 2(1H) - pyrimidinone in 20 ml. of methanol is treated with 160 mg. (4.0 millimoles) of sodium hydroxide and 2 ml. of water. The mixture is refluxed for 45 minutes and the solution is concentrated at reduced pressure. The residue is dissolved in 20 ml. of water and small portions of Dowex 50×4 (H+) resin are added until the pH of the solution is 4.0. The resin and precipitated benzoic acid are removed, and washed well with water. The combined filtrates are extracted with six 25-ml. portions of ether. The aqueous layer is concentrated at reduced pressure and the residue (300 mg.) in 5 ml. of methanol is treated with 1 ml. of ether. The precipitated solid is removed and the filtrate is concentrated to 0.3 ml. and kept at 5°C. for 18 hours. The solid (107 mg., m.p. 196—205°C). obtained, when crystallized from 0.5 ml. of methanol and 0.5 ml. of ether gives 74 mg. (27%) of 5 - fluoro - 2′ - C - methyluridine: m.p. 205—207°C.

When the 1 - (2′, 3′, 5′ - tri - O - benozyl - 2′ - C - methyl - β - D - ribofuranosyl) - 5 - fluoro - 4 - methoxy - 2(1H) - pyrimidinone in the above procedure is replaced by an equivalent amount of 1 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - ethyl - β - D - ribofuranosyl) - 5 - fluoro - 4 - methoxy - 2(1H) - pyrimidinone, 1 - 2′, 3′, 5′ - tri - O - benzoyl - 3′ - C - methyl - β - D - ribofuranosyl) -5 - fluoro - 4 - methoxy - 2(1H) - pyrimidinone, or 1 - (2′, 3′, 5′ - tri - O - benzoyl - 3′ - C - ethyl - β - D - ribofuranosyl) - 5 - fluoro - 4 - methoxy - 2(1H) - pyrimidinone there is obtained respectively 5 - fluoro - 2′ - C - ethyluridine, 5 - fluoro - 3′ - C - methyluridine, or 5 - fluoro 3′ - C - ethyluridine.

Example 12

Step A

1 - (2′, 3′, 5′ - Tri - O - benzovl - 2′ - C - methyl - β - D - ribofuranosvl) - 5 - methyl - 4 - methoxy - 2(1H) - pyrimidinone

A solution of 4.8 g. (9.7 millimoles) of 2, 3, 5 - tri - O - benzoyl - 2 - C - methyl - β - D - ribofuranosyl chloride in 18 ml. of dry toluene is added to 3.5 g. (22 millimoles) of 2, 4 - dimethoxy - 5 - methyl - pyrimidine and the mixture is refluxed for 96 hours. The toluene solution is concentrated, the residue is then dissolved in 100 ml. of ether, extracted with three 50-ml. portions of 4 N hydrochloric acid and three 50-ml. portions of saturated sodium hydrogen carbonate and finally with water. The ethereal solution is concentrated and the residue is in a 19:1 mixture of benzene and ethyl acetate. After the elution of several by-products, fractions containing the product are obtained. Crystallization from benzene and ether gives 1 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - methyl - β - D - ribofuranosyl) - 5 - methyl - 4 - methoxy - 2(1H) - pyrimidinone.

When the 2, 3, 5 - tri - O - benzoyl - 2 - C - methyl - β - D - ribofuranosyl chloride in the above procedure is replaced by 2, 3, 5 - tri - O - benzoyl - 2 - C - ethyl - β -D - ribofuranosyl chloride, 2, 3, 5 - tri - O - benzoyl - 3 - C - methyl - β - D - ribofuranosyl chloride, or 2, 3, 5 - tri - O - benzoyl - 3 - C - ethyl - β - D - ribofuranosyl chloride there is obtained respectively 1 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - ethyl - β - D - ribofuranosyl) - 5 - methyl - 4 - methoxy - 2(1H) - pyrimidinone, 1 - (2′, 3′, 5′ - tri - O - benzoyl - 3 - C - methyl - β - D - ribofuranosyl) - 5 - methyl - 4 - methoxy - 2(1H) - pyrimidinone, or 1 - (2′, 3′, 5′ - tri - O - benzoyl - 3′ - C - ethyl - β - D - ribofuranosyl) - 5 - methyl - 4 - methoxy - 2(1H) - pyrimidinone.

Step B

5 - Methyl - 1 - (2′ - C - methyl - β - D - ribofuranosyl)uracil, i.e. 5 - methyl - 2′ - C - methyluridine

A suspension of 602.5 mg. (1.0 millimole) of 1 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - methyl - β - D-ribofuranosyl) - 5 - methyl - 4 - methoxy - 2(1H) - pyrimidinone in 20 ml. of methanol is treated with 160 mg. (4.0 millimoles) of sodium hydroxide and 2 ml. of water. The mixture is refluxed for 45 minutes and the solution is concentrated at reduced pressure. The residue is dissolved in 20 ml. of water and small portions of Dowex 50×4 (H+) resin are added until the pH of the solution is 4.0. The resin and precipitated benzoic acid are removed and washed well with water; the combined filtrates are extracted with six 25-ml. portions of ether. The aqueous layer is concentrated at reduced pressure and the residue in methanol is treated with ether. The precipitated solid is removed; the filtrate is concentrated and a residual solid containing 5 - methyl - 2′ - C - methyluridine is obtained.

When the 1 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - methyl - β - D - ribofuranosyl) - 5 - methyl - 4 - methoxy - 2(1H) - pyrimidinone in the above procedure is replaced by an equivalent amount of 1 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - ethyl - β - D - ribofuranosyl) - 5 - methyl - 4 - methoxy - 2(1H) - pyrimidinone, 1 - (2′, 3′, 5′ - tri - O - benzoyl - 3′ - C - methyl - β - D - ribofuranosyl) - 5 - methyl - 4 - methoxy - 2(1H) - pyrimidinone, or

ethyl - β - D - ribofuranosyl) - 5 - methyl - 4 - methoxy - 2(1H) - pyrimidinone there is obtained respectively 5 - methyl - 2′ - C - ethyluridine, 5 - methyl - 3′ - C - methyluridine, or 5 - methyl - 3′ - C - ethyluridine.

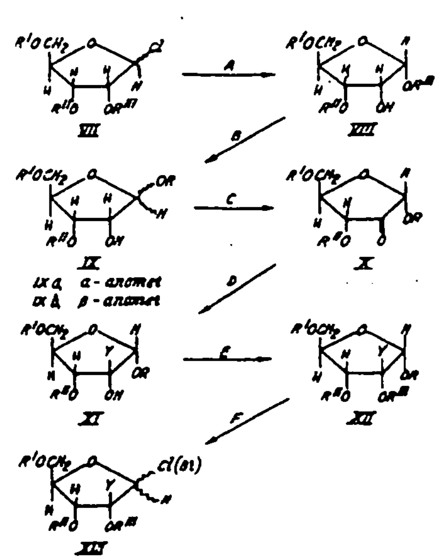
EXAMPLE 13

5 - Methyl - 1 - (2′ - C - methyl - β - D - ribofuranosyl)cytosine [5 - methyl - 2′ - C - methylcytidine]

A solution of 80 mg. (0.13 millimole) of 1 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - methyl - B - D - ribofuranosyl) - 5 - methyl - 4 - methoxy - 2(1H) - pyrimidinone in 7 ml. of methanol, saturated with ammonia at 0°C., is heated at 100°C. in a sealed tube for 18 hours. The reaction solution is concentrated at reduced pressure. The residue is then dissolved in 10 ml. of water and extracted with three 5-ml. portions of ether. The aqueous phase is concentrated at reduced pressure; the residue, when crystallized from methanol and ether, gives 5 - methyl - 2′ - C - methylcytidine.

When the 1 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - methyl - β - D - ribofuaanosyl) - 5 - methyl - 4 - methoxy - 2(1H) - pyrimidinone in the above procedure is replaced by an equivalent amount of 1 - (2′, 3′, 5′ - tri - O - benzoyl - 2′ - C - ethyl - β - D - ribofuranosyl) - 5 - methyl - 4 - methoxy - 2(1H) - pyrimidinone, 1 - (2′, 3′, 5′ - tri - O - benzoyl - 3′ - C - methyl - β - D - ribofuranosyl) - 5 - methyl - 4 - methoxy - 2(1H) - pyrimidinone, or 1 - (2′, 3′, 5′ - tri - O - benzoyl - 3′ - C - ethyl - β - D - ribofuranosyl) - 5 - methyl - 4 - methoxy - (2(1H) - pyrimidinone there is obtained respectively 5 - methyl - 2′ - C - ethylcytidine, 5 - methyl - 3′ - C - methylcytidine, or 5 - methyl - 3′ - C - ethylcytidine.

The 2-alkylribofuranosyl halides used as starting materials in the process of the present invention may be obtained by the reactions shown in the following scheme, which are described in more detail below.



where R′, R″ and R‴ are C_{1-6} alkanoyl, benzoyl or substituted benzoyl groups and each of R and Y is a C_{1-5} alkyl radical.

Apart from the compounds mentioned above, the following 2 - C - methyl compounds can also be obtained by this procedure: 2, 3, 5 - tri - O - acetyl - 2 - C - methyl - D - ribofuranosyl chloride, 2, 3, 5 - tri - O - propionyl - 2 - C - methyl - D - ribofuranosyl chloride, 2, 3, 5 - tri - O - butyryl - 2 - C - methyl - D - ribofuranosyl chloride, 2, 3, 5 - tri - O - benzoyl - 2 - C - methyl - D - ribofuranosyl chloride, 2, 3, 5 - tri - O - toluyl - 2 - C - methyl - D - ribofuranosyl chloride, 2, 3, 5 - tri - O - xyloyl - 2 - C - methyl - D - ribofuranosyl chloride, 2, 3, 5 - tri - O - methoxybenzoyl - 2 - C - methyl - D - ribofuranosyl chloride, 2, 3, 5 - tri - O - chlorobenzoyl - 2 - C - methyl - D - ribofuranosyl chloride, 2, 3, 5 - tri - O - acetyl - 2 - C - methyl - D - ribofuranosyl bromide, 2, 3, 5 - tri - O - benzoyl - 2 - C - methyl - D - ribofuranosyl bromide, 2, 3, 5 - tri - O - p - nitrobenzoyl - 2 - C - methyl - D - ribofuranosyl bromide and 2, 3, 5 - tri - O - p - nitrobenzoyl - 2 - C - methyl - D - ribofuranosyl chloride.

In step A of this process, a 2, 3, 5 - tri - O - acyl - D - ribofuranosyl halide (VII) in which the acyl group is C_{1-6} alkanoyl, benzoyl or benzoyl substituted by one or more halogen atoms or C_{1-5} alkyl, C_{1-5} alkoxy or nitro groups is converted into the 1, 3, 5 - tri - O - acyl - α - D - ribofuranose (VIII) by adding an aqueous acetone solution to the halogenose (VII) and permitting the mixture to stand for about an hour at a temperature from 5° to 50°C. The speed of the reaction will be greater at the high temperature. A solution of approximately 4 to 6 parts (v/v) of water in acetone is preferred, although the concentration is not critical. A greater proportion of water will tend to hydrolyse the halogen atom at the 1-position also, giving a more unfavourable mixture of products. The product is suitably recovered by methods known in the art.

In Step B, the 1, 3, 5 - tri - O - acyl - α - D - ribofuranose (VIII) is treated with HCl and a C_{1-5} alkanol to obtain the corresponding alkyl 3, 5 - di - O - acyl - α - (and β) - D - ribofuranosides (IX). This reaction suitably takes place at a temperature of from 5° to 50°C, a temperature of 25°C being preferred. Other mineral acids such as HBr or sulphuric acid may also be used. Although methanol is preferred other C_{1-5} alkanols may be used. The α and β-anomers are separated by chromatography on silica gel.

The alkyl 3, 5 - di - O - acyl - α - D - ribofuranoside (IXa) is then oxidized to the alkyl 3,5 - di - O - acyl - α - D - *erythro*-pentofuran - 2 - uloside (X) in Step C. Suitable oxidizing agents are ruthenium tetroxide, chromium trioxide in pyridine, and dimethyl sulfoxide in acetic acid or acetic anhydride. Using ruthenium tetroxide, oxidation takes place conveniently at room temperature, although temperatures within the range of 5° to 50°C are suitable.

In Step D, the alkyl 3, 5 - di - O - acyl - α - D - *erythro*pentofuran - 2 - uloside (X) is reacted with a Grignard reagent in substantially stoichiometric proportions at a temperature range of from 5° to 80°C for a time period of a few minutes to several hours, thereby forming an alkyl 3, 5 - di - O - acyl - 2 - C - (C_{1-5} alkyl) - α - D - ribofuranoside (XI). Examples of Grignard reagents used in this reaction are methyl magnesium bromide, ethyl magnesium chloride and propyl magnesium iodide.

The alkyl 3, 5 - di - O - acyl - C - (C_{1-5} alkyl) - α - D - ribofuranoside (XI) is acylated to the alkyl 2, 3, 5 - tri - O - acyl - 2 - C - (C_{1-5} alkyl) - α - D - ribofuranoside (XII) in Step E at an elevated temperature. Temperatures within the range of 40° to 100°C are suitable, using as the acylating agent an acyl halide or an acid anhydride in the presence of an organic base such as pyridine, dimethylaniline or N-methylmorpholine, or an inorganic base such as sodium acetate in an inert solvent such as benzene, dioxane or tetrahydrofuran.

The alkyl 2, 3, 5 - tri - O - acyl - 2 - C - (C_{1-5} alkyl) - α - D - ribofuranside (XII) is converted to the halo sugar (XIII) in Step F by a halogen replacement reaction with the desired hydrogen halide in acetic acid. This replacement reaction takes place at a temperature of from 5° to 30°C for 5 to 24 hours.

These procedures are illustrated in the following Preparation.

Preparation of Starting Material

Step A

1, 3, 5 - Tri - O - benzoyl - α - ribofuranose

A solution of 2, 3, 5 - tri - O - benzoyl - D - ribofuranosyl chloride (prepared from 9.2 g. of acetyl 2, 3, 5 - tri - O - benzoyl - β - D - ribofuranoside) in 36 ml of acetone and 1.7 ml of water is kept at 25°C for 1 hour. The solution is diluted with 100 ml of methylene chloride and washed with 25 ml of cold 10 per cent sodium hydrogen carbonate solution. The organic phase is dried over anhydrous $MgSO_4$, and concentrated to a residual oil at reduced pressure. Addition of ether to the oil causes crystallization of 3.0 g. of 1, 3, 5 - tri - O - benzoyl - α - D - ribofuranose, m.p. 140—143°C.

Step B

Methyl 3, 5 - Di - O - benzoyl - α(and β) - D - ribofuranoside

A solution of 5 g. of 1, 3, 5 - tri - O - benzoyl - α - D - ribofuranose in 1000 ml of one percent methanolic hydrogen chloride is kept at 25°C for 4 hours. The hydrogen chloride is neutralized with solid sodium hydrogen carbonate and the mixture is filtered. The filtrate is concentrated to dryness at reduced pressure, and the residue is leached with two 250-ml. portions of methylene chloride. The methylene chloride extracts are combined and concentrated at reduced pressure. The residual oil is chromatographed on silica gel in a mixture of benzene and ethyl acetate (4:1). After elution of some unreacted starting material, several fractions containing methyl 3, 5 - di - O - benzoyl - β - D - ribofuranoside are obtained. The product is isolated as a crystalline solid by concentration of the solution to dryness.

Concentration of later column fractions gives a residual oil contacting mostly methyl 3, 5 - di - O - benzoyl - α - D - ribofuranoside.

Step C

Methyl 3, 5 - Di - O - benzoyl - α - D - *erythro*pentofuran - 2 - uloside

A solution of 12.5 g. of sodium metaperiodate in 150 ml of water is cooled in an

ice bath and added portionwise to a vigorously stirred suspension of 1.5 g. of ruthenium dioxide in 150 ml. of carbon tetrachloride cooled in an ice bath. About 20 to 30 minutes after the addition is complete, most of the black insoluble ruthenium dioxide has been converted to a solution of yellow ruthenium tetroxide. The carbon tetrachloride layer is separated from the water and added over 15 minutes to a stirred solution of 2.2 g. (6 millimoles) of methyl 3, 5 - di - O - benzoyl - α - D - ribofuranoside in 150 ml. of carbon tetrachloride. After one hour, the reaction mixture, which now contains a black precipitate of ruthenium dioxide, is warmed to room temperature and stirred for an additional 2 hours. The course of the reaction may be followed by thin layer chromatography on silica gel in benzene-ethyl acetate (4:1).

The reaction mixture is treated with 1 ml. of isopropanol in 5 ml. of carbon tetrachloride to decompose unreacted ruthenium tetroxide. The black ruthenium dioxide is removed and washed with water; the combined filtrates are washed with 10 ml. of saturated sodium hydrogen carbonate solution. The carbon tetrachloride layer is concentrated at reduced pressure, and a residue containing methyl 3, 5 - di - O - benzoyl - α - D - *erythro*-pentofuran - 2 - uloside is obtained. The product is purified by a combination of chromatography on silica gel followed by crystallization from ether-petroleum ether.

Step D

Methyl 3, 5 - Di - O -benzoyl - 2 - C - ethyl - α - D - ribofuranoside

A solution of ethyl magnesium iodide is prepared by adding 3.2 g. (21 millimoles) of ethyl iodide in 50 ml. of dry ether to a stirred suspension of 0.64 g. (26.4 millimoles) of magnesium shavings in 10 ml. of dry ether. The Grignard solution is added to a stirred solution of 0.98 g. (2.64 millimoles) of methyl 3, 5 - di - O - benzoyl - α - D - *erythro*-pentofuran - 2 - uloside in 60 ml.of dry ether at 5°C. A heavy white precipitate forms immediately. The reaction mixture is poured into a cold stirred mixture of 8 ml. of ether and 10 g. of ammonium chloride dissolved in 120 ml. of water. The water layer is separated and extracted with three 40-ml. portions of ether. The ethereal layers are combined, washed with 20 ml. of saturated sodium chloride solution, and dried over anhydrous magnesium sulphate. Concentration of the ethereal solution at reduced pressure gives a residue containing methyl 3, 5 - di - O - benzoyl - 2 - C - ethyl - α - D - ribofuranoside. The product is purified by chromatography on silica gel in benzene-ethyl acetate (4:1).

Step E

Methyl 2, 3, 5 - tri - O - benzoyl - 2- C - ethyl - α - D - ribofuranoside

To 6.1 g. (15.3 millimoles) of methyl 3, 5 - di - O - benzoyl - 2 - C - ethyl - α - D - ribofuranoside in 50 ml. of dry (BaO) pyridine is added 4.4 g. (30.6 millimoles) of benzoyl chloride and the mixture is heated at 90 ± 5°C for 16 hours. The mixture is cooled to 25°C stirred with 5 ml. of water for 30 minutes, and added to 250 ml. 10% hydrochloric acid and 300 ml. of chloroform. The aqueous layer is extracted with two 250 ml. portions of chloroform, and the combined chloroform layers are washed with two 200-ml. portions of saturated sodium hydrogen carbonate solution and 300 ml. of saturated solution. After being dried over anhydrous magnesium sulphate, the chloroform solution is concentrated at reduced pressure and the residual oil is chromatographed on silica gel in chloroform. Purified methyl 2, 3, 5 - tri - O - benzyl - 2 - C - ethyl - α - D - ribofuranoside is obtained after removal of the solvent from early column fractions.

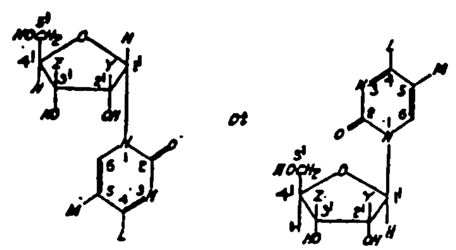
Step F

2, 3, 5 - Tri - O - benzoyl - 2 - C - ethyl - β - D - ribofuranosyl bromide

A solution of 2.6 millimoles of methyl 2, 3, 5 - tri - O - benzoyl - 2 - C - ethyl - α - D - ribofuranoside in 7.5 ml. of acetic acid is treated with 0.25 ml. of acetyl bromide and 7.5 ml. of 32% (w/w) solution of hydrogen bromide in acetic acid. The mixture is kept at 25°C for 24 hours. The mixture is concentrated and a portion of dry toluene is distilled at reduced pressure from the residue to remove excess hydrogen bromide and acetic acid. The residue is dissolved in dry ether and quickly washed with cold saturated sodium bicarbonate and finally with cold water. After being dried, the ethereal solution is concentrated and a residue of 2, 3, 5 - tri - O - benzoyl - 2 - C - methyl - β - D - ribofuranosyl bromide is obtained.

WHAT WE CLAIM IS:—

1. A compound having the formula:



where L is C_{1-5} alkoxy, hydroxy, amino, or (C_{1-5} alkyl)-substituted amino; M is hydrogen, C_{1-5} alkyl, halogen, C_{1-5} halogenated alkyl, C_{1-5} alkoxy, hydroxy, amino or (C

alkyl)-substituted amino and Y is hydrogen and Z is C_{1-5} alkyl or Y is C_{1-5} alkyl and Z is hydrogen.

2. A compound as claimed in claim 1, in which Z is methyl.

3. A compound as claimed in claim 1, in which Y is methyl.

4. A compound as claimed in claim 1, 2 or 3, in which L is hydroxy.

5. A compound as claimed in claim 1, 2 or 3, in which L is amino.

6. A compound as claimed in any one of claims 1—5, in which M is trifluoromethyl.

7. A compound as claimed in claim 1, in which L is amino, M and Z are hydrogen and Y is methyl.

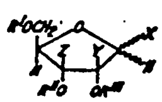
8. A compound as claimed in claim 1, in which L is amino, M and Y are hydrogen and Z is methyl.

9. A compound as claimed in claim 1, in which L is amino, M is fluoro, Y is methyl and Z is hydrogen.

10. A compound as claimed in claim 1, in which L is hydroxy, M is fluoro, Y is methyl and Z is hydrogen.

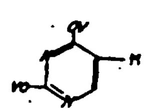
11. A compound as claimed in claim 1, in which L is hydroxy, M and Z are hydrogen and Y is methyl.

12. The process that comprises (A) reacting a ribofuransoyl halide compound of the formula:



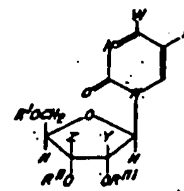
(C)

where each of R′, R″ and R‴ is an acyl group and X is halogen, with a compound of the formula:



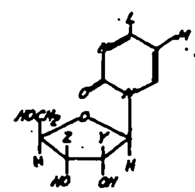
(III)

where V is hydrogen on C_{1-5} alkyl and M is as defined in claim 1, or a mercury complex thereof to form an intermediate of the formula:



(IV)

where R, R″ and R‴ are acyl, M, Z and Y are as defined in claim 1 and W is C_{1-5} alkoxy or hydroxy and (B) subjecting compound IV to (a) reaction with a solvent in the presence of a base when W in Compound IV is hydroxy; or (b) reaction with ammonia, a C_{1-5} alkylamine or a di(C_{1-5} alkyl)amine when W in Compound IV is alkoxy; to produce a compound of the formula:



(V)

where L, M, Y and Z are as defined in claim 1.

13. A process as claimed in claim 12, as applied to the production of a compound as claimed in any one of claims 2—11.

14. A process as claimed in claim 12 or 13, in which all the steps are carried out in an inert solvent medium at a temperature of from 25°C to 60°C.

15. A process as claimed in claim 12, substantially as hereinbefore described in any one of the foregoing Examples.

16. A process as claimed in any one of claims 12—15, including the step of preparing the ribofuranosyl halide compound by a process claimed in the specification of our copending application No. 49923/66 (1163102).

17. A compound as claimed in claim 1, when prepared by a process as claimed in any one of claims 12—16 or an obvious chemical equivalent of such a process.

For the Applicants,
D. YOUNG & CO.,
Chartered Patent Agents,
9, Staple Inn,
London, W.C.1.

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